

CLAIMS

What is claimed is:

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1. A polypeptide having an activity to transfer fucose to an N-acetylglucosamine residue in an N-acetyllactosamine ($\text{Gal } \beta \text{ 1-4GlcNAc}$) structure existing in a nonreducing terminus of a sugar chain via an $\alpha \text{ 1,3-linkage}$, but not having an activity to transfer fucose to an N-acetylglucosamine residue in an $\alpha \text{ 2,3-sialyl N-acetyllactosamine (NeuAc } \alpha \text{ 2-3Gal } \beta \text{ 1-4GlcNAc)}$ structure existing in a nonreducing terminus of a sugar chain via an $\alpha \text{ 1,3-linkage}$.
 2. A polypeptide selected from the following (a), (b) and (c):
 - (a) a polypeptide comprising the amino acid sequence represented by SEQ ID NO: 1 or 2,
 - (b) a polypeptide comprising amino acid sequence of residues 56 to 359 represented by SEQ ID NO: 1 or 2,
 - (c) a polypeptide comprising an amino acid sequence wherein one or more amino acids are deleted, substituted, or added in the amino acid sequence of the polypeptide of (a) or (b), having an activity to transfer fucose to an N-acetylglucosamine residue in an N-acetyllactosamine ($\text{Gal } \beta \text{ 1-4GlcNAc}$) structure existing in a nonreducing terminus of a sugar chain via an $\alpha \text{ 1,3-linkage}$, but not having an activity to transfer fucose to the $\alpha \text{ 2,3-sialyl N-acetyllactosamine (NeuAc } \alpha \text{ 2-3Gal } \beta \text{ 1-4GlcNAc)}$ structure existing in a nonreducing terminus of a sugar chain via an $\alpha \text{ 1,3-linkage}$.
 3. The polypeptide according to claim 1 or 2, wherein the activity of transferring fucose to an N-acetylglucosamine residue in the $\text{Gal } \beta \text{ 1-4GlcNAc}$ structure existing in a nonreducing terminus of a sugar chain via an $\alpha \text{ 1,3-linkage}$ is the Lewis x sugar chain [$\text{Gal } \beta \text{ 1-4(Fuc } \alpha \text{ 1-3)GlcNAc}$] and the Lewis y sugar chain
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[Fuc α 1-2Gal β 1-4(Fuc α 1-3)GlcNAc] synthesizing activity, and the activity of transferring fucose to an N-acetylglucosamine residue in the NeuAc α 2-3Gal β 1-4GlcNAc structure existing in a nonreducing terminus of a sugar chain via an α 1,3-linkage is the sialyl Lewis x sugar chain [NeuAc α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc] synthesizing activity.

4. A DNA selected from the following (a), (b), (c), (d), (e), (f), (g) and (h):
- (a) a DNA encoding the polypeptide selected from the polypeptides according to claims 1, 2 and 3,
 - (b) a DNA having nucleotides 280 to 1194 of a nucleotide sequence represented by SEQ ID NO: 3,
 - (c) a DNA having nucleotides 115 to 1194 of a nucleotide sequence represented by SEQ ID NO: 3,
 - (d) a DNA having nucleotides 1454 to 2368 of a nucleotide sequence represented by SEQ ID NO: 4,
 - (e) a DNA having nucleotides 1289 to 2368 of a nucleotide sequence represented by SEQ ID NO: 4,
 - (f) a DNA having nucleotides 460 to 1374 of a nucleotide sequence represented by SEQ ID NO: 5,
 - (g) a DNA having nucleotides 295 to 1374 of a nucleotide sequence represented by SEQ ID NO: 5, and
 - (h) a DNA hybridizing under stringent conditions with DNA selected from (a), (b), (c), (d), (e), (f) and (g); and said DNA encodes a polypeptide having an activity to transfer fucose to an N-acetylglucosamine residue in an N-acetylglucosamine (Gal β 1-4GlcNAc) structure existing in a nonreducing terminus of a sugar chain via an α 1,3-linkage, but not having an activity to transfer fucose to an α 2,3-sialyl N-acetylglucosamine (NeuAc α 2-3Gal β 1-4GlcNAc) structure existing in a nonreducing terminus of a sugar chain via an α 1,3-linkage.

5. A recombinant DNA obtained by integrating the DNA according to claim 4 into a vector.

6. The recombinant DNA according to claim 5 wherein it is plasmid pAMo-mFT9 or plasmid pBS-hFT9 (S2).

7. A transformant having the recombinant DNA according to claim 5 or 6.

8. The transformant according to claim 7 wherein it is a transformant selected from microorganisms, animal cells, plant cells, insect cells, non-human transgenic animals, and transgenic plants.

9. The transformant according to claim 8 wherein the microorganism belongs to *Escherichia*.

10. The transformant according to claim 8 wherein the animal cell is selected from mouse myeloma cells, rat myeloma cells, mouse hybridoma cells, CHO cell, BHK cell, African green monkey kidney cells, Namalwa cell, Namalwa KJM-1 cell, human fetal kidney cells, and human leukemia cells.

11. The transformant according to claim 8 wherein the insect cell is selected from *Spodoptera frugiperda* ovarian cells, *Trichoplusia ni* ovarian cells, and silkworm ovarian cells.

12. A method for producing a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises culturing in a medium a transformant having a recombinant DNA obtained by inserting a DNA encoding the polypeptide into a vector; producing and accumulating said polypeptide in said medium; and collecting said polypeptide from said medium.

13. A method for producing a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises feeding a non-human transgenic animal having a recombinant DNA obtained by inserting a DNA encoding the polypeptide into a vector; producing and accumulating said polypeptide in said non-human transgenic animal; and collecting said polypeptide from said non-human transgenic animal.

14. The method for producing the polypeptide according to claim 13, wherein the production and accumulation of said polypeptide is carried out in the milk of said non-human transgenic animal.

15. A method for producing a polypeptide selected from the polypeptides according to claim 1, 2 and 3, which comprises growing a transgenic plant having a recombinant DNA obtained by inserting a DNA encoding the polypeptide into a vector; producing and accumulating said polypeptide in said transgenic plant; and collecting said polypeptide from said transgenic plant.

16. A method for producing a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises using a DNA encoding the polypeptide, and synthesizing said polypeptide by an *in vitro* transcription-translation system.

17. A method for producing a reaction product wherein fucose is added to an N-acetylglucosamine residue in the N-acetylglucosamine structure of an acceptor substrate via an α 1,3-linkage, using a polypeptide selected from the polypeptides according to claims 1, 2 and 3 as an enzyme source; which comprises placing the following (a), (b) and (c) in an aqueous medium:

(a) said enzyme source,

(b) an acceptor substrate selected from: (i) N-acetylglucosamine(Gal β 1-4GlcNAc),

(ii) oligosaccharides having the N-acetylglucosamine structure in a nonreducing

terminus thereof, (iii) complex carbohydrates having the N-acetylactosamine structure in a nonreducing terminus of sugar chains, (iv) their derivatives wherein the N-acetylactosamine structure is modified by sulfate group, and (v) their derivatives wherein the N-acetylactosamine structure is modified by sugar, but a galactose residue in the N-acetylactosamine structure is not modified by sialic acid via an α 2,3-linkage, and

(c) guanosine-5' -diphosphate fucose;

producing and accumulating the reaction product, in the aqueous medium; and

collecting said reaction product from said aqueous medium.

18. The method for producing the reaction product according to claim 17 wherein a derivative is selected from sugar chains having, in a nonreducing terminus thereof, any one of the following oligosaccharide structures: Fuc α 1-2Gal β 1-4GlcNAc, Gal α 1-3Gal β 1-4GlcNAc, Gal α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc, GalNAc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc, Gal α 1-4Gal β 1-4GlcNAc, Gal β 1-4GlcNAc(6SO₃); and complex carbohydrates containing said sugar chains.

19. A method for producing a reaction product wherein fucose is added to a glucose residue in a lactose structure of an acceptor substrate via an α 1,3-linkage, using a polypeptide selected from the polypeptides according to claim 1, 2 and 3 as an enzyme source; which comprises placing the following (a), (b) and (c) in an aqueous medium:

(a) said enzyme source,

(b) an acceptor substrate selected from (i) lactose (Gal β 1-4Glc), (ii)

oligosaccharides having a lactose structure in a nonreducing terminus thereof,

(iii) complex carbohydrates having a lactose structure in a nonreducing terminus of sugar chains, (iv) their derivatives wherein a lactose structure is modified by sulfate group, and (v) their derivatives wherein a lactose structure is modified by sugar(s), but a galactose residue in the lactose structure is not

modified by sialic acid via an α 2,3-linkage, and
(c) guanosine-5' -diphosphate fucose;
producing and accumulating the reaction product, in said aqueous medium; and
collecting said reaction product from said aqueous medium.

20. The method for producing the reaction product according to claim 19 wherein a derivative is selected from sugar chains having, in a nonreducing terminus thereof, any one of the following oligosaccharide structures: Gal α 1-3Gal β 1-4Glc, Gal α 1-3(Fuc α 1-2)Gal β 1-4Glc, GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc, Gal α 1-4Gal β 1-4Glc, Gal β 1-4Glc(6SO₃); and complex carbohydrates containing said sugar chains.

21. A method for producing a sugar chain having a structure wherein fucose is added to an N-acetylglucosamine residue or a glucose residue via an α 1,3-linkage, or a complex carbohydrate containing said sugar chain; which comprises, culturing in a medium a transformant selected from the transformants derived from microorganisms, animal cells, plant cells, and insect cells according to claim 8; producing and accumulating the sugar chain or the complex carbohydrate in said medium; and
collecting said sugar chain or said complex carbohydrate from said medium.

22. A method for producing a sugar chain having structure wherein fucose is added to an N-acetylglucosamine residue or a glucose residue via an α 1,3-linkage, or a complex carbohydrate containing said sugar chain; which comprises, feeding a non-human transgenic animal according to claim 8; producing and accumulating the sugar chain or the complex carbohydrate in the non-human transgenic animal; and
collecting said sugar chain or said complex carbohydrate from said non-human transgenic animal.

23. A method for producing a sugar chain having a structure wherein fucose is added to an N-acetylglucosamine residue or a glucose residue via an α 1,3-linkage, or a complex carbohydrate containing said sugar chain; which comprises, growing a transgenic plant according to claim 8;
producing and accumulating the sugar chain or the complex carbohydrate in said transgenic plant; and
collecting said sugar chain or said complex carbohydrate from said transgenic plant.
24. The production method according to any one of claims 17 to 23 wherein the complex carbohydrate is selected from glycoproteins, glycolipids, proteoglycans, glycopeptides, lipopolysaccharides, peptideglycans and glycosides wherein a sugar chain binds to compounds such as steroids.
25. The method for producing the sugar chain or the complex carbohydrate according to claim 22 wherein the generation and accumulation of said sugar chain or said complex carbohydrate is carried out in the milk of said non-human transgenic animal.
26. A method for determining the expression level of a gene encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3, by hybridization using DNA encoding the polypeptide.
27. An oligonucleotide selected from the following oligonucleotides:
an oligonucleotide having an identical sequence to 10 to 50 contiguous nucleotides in a nucleotide sequence of DNA selected from a DNA encoding the polypeptide selected from the polypeptides according to claims 1, 2 and 3; a DNA having the nucleotide sequence represented by SEQ ID NO: 3; a DNA having the nucleotide sequence represented by SEQ ID NO: 4; and a DNA having the nucleotide sequence

represented by SEQ ID NO. 5; and,
an oligonucleotide having a complementary sequence to said oligonucleotide and
a derivative of each of said oligonucleotides.

28. The oligonucleotide according to claim 27, wherein said oligonucleotide derivative is selected from the following oligonucleotide derivatives: an oligonucleotide derivative obtained by converting a phosphodiester bond into a phosphorothioate bond in an oligonucleotide; an oligonucleotide derivative obtained by converting a phosphodiester bond into a N3'-P5' phosphoramidate bond in an oligonucleotide; an oligonucleotide derivative obtained by converting a ribose and phosphodiester bond into a peptide-nucleic-acid bond in an oligonucleotide; an oligonucleotide derivative obtained by substituting uracil with C-5 propynyl uracil in an oligonucleotide; an oligonucleotide derivative obtained by substituting uracil with C-5 thiazolyl uracil in an oligonucleotide; an oligonucleotide derivative obtained by substituting cytosine with C-5 propynylcytosine in an oligonucleotide; an oligonucleotide derivative obtained by substituting cytosine with phenoxazine-modified cytosine in an oligonucleotide; an oligonucleotide derivative obtained by substituting ribose with 2'-O-propylribose in a DNA; and an oligonucleotide derivative obtained by substituting ribose with 2'-methoxyethoxyribose in the oligonucleotide.

29. A method for determining the expression level of a gene encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3 by polymerase chain reaction, using the oligonucleotide according to claim 27 or 28.

30. A method for detecting encephalopathy, renal diseases and cancers, using the method according to claim 26 or 29.

31. A method for suppressing the transcription of a DNA encoding a polypeptide

selected from the polypeptides according to claims 1, 2 and 3, using a DNA selected from a DNA encoding the polypeptide, a DNA having the nucleotide sequence represented by SEQ ID NO: 3, a DNA having the nucleotide sequence represented by SEQ ID NO: 4, and a DNA having the nucleotide sequence represented by SEQ ID NO: 5.

32. A method for suppressing the translation of an mRNA encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3, using a DNA selected from a DNA encoding the polypeptide, a DNA having the nucleotide sequence represented by SEQ ID NO: 3, a DNA having the nucleotide sequence represented by SEQ ID NO: 4, and a DNA having the nucleotide sequence represented by SEQ ID NO: 5.

33. A method for suppressing the transcription of a DNA encoding the polypeptide according to claim 1, 2 or 3, using the oligonucleotide according to claim 27 or 28.

34. A method for suppressing the translation of an mRNA encoding the polypeptide according to claim 1, 2 or 3, using the oligonucleotide according to claim 27 or 28.

35. An antibody recognizing a polypeptide selected from the polypeptides according to claims 1, 2 and 3.

36. An immunoassay which detects a polypeptide selected from the polypeptides according to claims 1, 2 and 3, using the antibody according to claim 35.

37. An immunohistological staining method which detects a polypeptide selected from the polypeptides according to claims 1, 2 and 3, using the antibody according to claim 35.

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38. A reagent for immunohistological staining which contains the antibody of claim 35.
39. An agent for diagnosing encephalopathy, renal diseases and cancers, which contains the antibody of claim 35.
40. A method for screening a substance that changes the activity of a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises contacting said polypeptide with test samples.
41. A method for screening a substance that changes the expression of a gene encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises contacting a cell expressing said polypeptide with test samples, and measuring the amount of the Lewis x or Lewis y sugar chain using an anti-Lewis x or anti-Lewis y antibody.
42. A method for screening a substance that changes the expression of a gene encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3, which comprises contacting a cell expressing said polypeptide with test samples, and measuring the amount of said polypeptide using the antibody of claim 35.
43. A promoter DNA for the transcription of a gene encoding a polypeptide selected from the polypeptides according to claims 1, 2 and 3.
44. The promoter DNA according to claim 43 which functions in a cell selected from neurons, kidney cells, gastric epithelium cells, leukocyte cells, cerebral tumor cells, neuroblastoma cells, melanoma cells, renal cancer cells, stomach cancer cells, colon cancer cells, and pancreatic cancer cells.
45. The promoter DNA according to claim 43 or 44 which is derived from human or

mouse.

46. A method for screening a substance that changes the efficiency of transcription by a promoter DNA selected from the promoter DNAs according to claims 43, 44 and 45, which comprises transforming an animal cell with a plasmid comprising the promoter DNA and a reporter gene ligated downstream of said promoter DNA; contacting transformant with a test sample; and measuring the amount of the translation product of said reporter gene.
47. The screening method according to claim 46 wherein the reporter gene is a gene selected from chloramphenicol acetyltransferase genes, β -galactosidase genes, luciferase genes and green fluorescent protein genes.
48. A non-human knockout animal wherein a DNA encoding the polypeptide selected from the polypeptides according to claims 1, 2 and 3 is deleted or mutated.
49. The non-human knockout animal according to claim 48 wherein the non-human knockout animal is a mouse.

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